EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent:
07.11.2012 Bulletin 2012/45

(21) Application number: 05823930.2

(22) Date of filing: 21.12.2005

(51) Int Cl.:
A61K 31/44 (2006.01) A61K 31/445 (2006.01)
A61K 31/495 (2006.01) A61K 31/496 (2006.01)
A61K 31/404 (2006.01)

(54) INDOLIDONE DERIVATIVES FOR THE TREATMENT OR PREVENTION OF FIBROTIC DISEASES
INDOLIDON-DERIVATE ZUR BEHANDLUNG ODER PRÄVENTION FIBROTISCHER KRANKHEITEN
MEDICAMENTS POUR LE TRAITEMENT OU LA PREVENTION DE MALADIES FIBROTIQUES

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LI LT LU LV MC NL PL PT RO SE SI SK TR
Designated Extension States:
BA HR YU


(43) Date of publication of application: 12.09.2007 Bulletin 2007/37

(60) Divisional application:
10184949.5 / 2 384 751

(73) Proprietors:
• Boehringer Ingelheim International GmbH
55216 Ingelheim am Rhein (DE)
Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LI LT LU LV MC NL PL PT RO SE SI SK TR
• Boehringer Ingelheim Pharma GmbH & Co.KG
55216 Ingelheim am Rhein (DE)
Designated Contracting States: DE

(72) Inventors:
• PARK, John, Edward
88447 Warthausen (DE)
• ROTH, Gerald, Jürgen
88400 Biberach (DE)
• HECKEL, Armin
88400 Biberach (DE)
• CHAUDHARY, Nveed
Middlesex HA9 8DG (DE)
• BRANDL, Trixi
CH-4056 Basel (CH)
• DAHMANN, Georg
88448 Attenweiler (DE)
• GRAUERT, Matthias
88400 Biberach (DE)

(74) Representative: Simon, Elke Anna Maria et al
Boehringer Ingelheim GmbH
Binger Strasse 173
55216 Ingelheim am Rhein (DE)

(56) References cited:
WO-A-00/56710 WO-A-01/27081
WO-A2-2004/017948

Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).
The present invention relates to a new use of an indolinone of general formula substituted in the 6 position, a tautomer, a diastereomer, an enantiomer, the mixtures thereof or a salt thereof, particularly a physiologically acceptable salt thereof.

BACKGROUND

Compounds of the above general formula I, the tautomers, the diastereomers, the enantiomers, the mixtures thereof and the salts thereof, particularly the physiologically acceptable salts thereof, have been described in WO 01/27081 and WO 04/13099 as having valuable pharmacological properties, in particular an inhibiting effect on various kinases, especially receptor tyrosine kinases such as VEGFR2, PDGFRα, PDGFRβ, FGFR1, FGFR3, EGFR, HER2, IGF1R and HGFR, as well as complexes of CDK's (Cyclin Dependent Kinases) such as CDK1, CDK2, CDK3, CDK4, CDK5, CDK6, CDK7, CDK8 and CDK9 with their specific cyclins (A, B1, B2, C, D1, D2, E, F, G1, G2, H, I and K) and to viral cyclin (cf. L. Mengtao in J. Virology 71(3), 1984-1991 (1997)), and on the proliferation of cultivated human cells, in particular endothelial cells, e.g. in angiogenesis, but also on the proliferation of other cells, in particular tumour cells.

However, none of these compounds have been described for their use in the treatment or prevention of the fibrotic diseases referred to in the present invention.

Remodeling is a normal response to tissue injury and inflammation that is observed in many tissues throughout the body. After resolution of the inflammation and repair of tissue damage, the tissue is generally returned to its original condition. Excessive uncontrolled tissue repair or the failure to stop remodeling when it is no longer required leads to a condition known as fibrosis. Fibrosis is characterized by excessive deposition of extracellular matrix components and overgrowth of fibroblasts. Fibrosis can occur in all tissues but is especially prevalent in organs with frequent exposure to chemical and biological insults including the lung, skin, digestive tract, kidney, and liver (Eddy, 1996, J Am Soc Nephrol, 7(12):2495-503; Dacic et al., 2003, Am J Respir Cell Mol Biol, 29S: S5-9; Wynn, 2004, Nat Rev Immunol, 4(8):583-94).

Fibrosis often severely compromises the normal function(s) of the organ and many fibrotic diseases are, in fact, life-threatening or severely disfiguring, such as idiopathic pulmonary fibrosis (IPF), liver cirrhosis, scleroderma, or renal fibrosis. Treatment options for these diseases are often limited to organ transplantation, a risky and expensive procedure.


Inhibition of PDGF attenuates both liver fibrosis and lung fibrosis in experimental models, suggesting fibrosis in different organs may have a common origin (Borkham-Kamphorst et al., 2004, Biochem Biophys Res Commun; Rice et al., 1999, Amer J Pathol, 155(1):213-221). An EGF receptor kinase inhibitor was also active in this lung fibrosis model.
Three-fold overexpression of an EGF family member, HB-EGF, in mouse pancreas islets was sufficient to cause development of fibrosis in both the exocrine and endocrine compartments (Means et al., 2003, Gastroenterology, 124 (4) : 1020-36).

[0008] Similarly, FGF1/FGF2-deficient mice show dramatically decreased liver fibrosis after chronic carbon tetrachloride (CC14) exposure (Yu et al., 2003, Am J Pathol, 163(4):1653-62). FGF expression is increased in human renal interstitial fibrosis where it strongly correlates with interstitial scarring (Strutz et al., 2000, Kidney Int, 57:1521-38) as well as in a model of experimental lung fibrosis (Barrios et al., 1997, Am J Physiol, 273 (2 Pt 1):L451-8), again lending credence to the idea that fibrosis in various tissues has a common basis.


[0011] As summarized above, several growth factors are upregulated in fibrosis and the inhibition of a single factor seems to reduce the severity of fibrosis in the fibrosis models.

SUMMARY OF THE INVENTION

[0012] Surprisingly, we found that a specific compound of above general formula I is effective in the treatment or prevention of a specific fibrotic disease.

[0013] The present invention thus relates to the compound 3-Z-[1-(4-N-((4-methyl-piperazin-1-yl)-methylcarbonyl)-N-methyl-amino)-anilino]-1-phenylmethylene]-6-methoxycarbonyl-2-indolinone (compound (u)), or a tautomer, adiastereomer, anenantiomer, the mixtures thereof or a salt thereof, for use in the prevention or treatment of idiopathic pulmonary fibrosis.

[0014] The present invention also relates to the monoethanesulfonate salt of the compound 3-Z-[1-(4-N-((4-methyl-piperazin-1-yl)-methylcarbonyl)-N-methyl-amino)-anilino]-1-phenylmethylene]-6-methoxycarbonyl-2-indolinone for use in the prevention or treatment of idiopathic pulmonary fibrosis.

DETAILED DESCRIPTION OF THE INVENTION

[0015] The above compound, (u), its tautomers, its stereoisomers or the physiologically acceptable salts thereof, as well as its manufacturing process, have been described in WO 01/27081.
BIOLOGICAL ACTIVITY

[0016] The following experimental results illustrate the present invention without representing a limitation of its scope.

Example B1:

[0017] In the following experiments of Example B1, Example A denotes the compound 3-Z-[1-(4-(N-dimethylaminoethylcarbonyl-N-methyl-amino)-anilino)-1-phenyl-methylene]-6-methoxycarbonyl-2-indolinone, which is further denoted as compound (m), and used here solely for comparison.

[0018] (A) Effect on lung morphology following bleomycin-induced pulmonary fibrosis.

Materials and Methods

[B009] Bleomycin sulfate (Bleomycin HEXAL™) was purchased from a local pharmacy.

Bleomycin administration and treatment protocols

[0020] All experiments were performed in accordance with German guidelines for animal welfare, performed by persons certified to work with animals and approved by the responsible authorities. Male Wistar rats were intratracheally injected with Bleomycin sulfate (10U/kg body weight in 300µl saline) or saline alone (saline control) using a catheter (0.5mm internal diameter, 1.0mm external diameter) through the nasal passage, following exposure to the anaesthetic Isoflurane for 5 minutes. The following day, the rats were orally treated with Example A (compound (m)) or saline suspended in 1ml 0.1% Natrosol. Control rats were administered 1ml 0.1% Natrosol (vehicle control).

[0021] A total of 25 rats were investigated and were grouped and treated as shown in Table 1.

<table>
<thead>
<tr>
<th>Intratracheal instillation</th>
<th>No. of animals</th>
<th>Compound</th>
<th>Treatment Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleomycin 10U /kg</td>
<td>10</td>
<td>Example A (Compound (m))</td>
<td>Days 1-21</td>
</tr>
<tr>
<td>Saline (300µl)</td>
<td>5</td>
<td>Vehicle only</td>
<td>Days 1-21</td>
</tr>
</tbody>
</table>

[0022] 21 days following bleomycin instillation, the rats were killed with a lethal intraperitoneal injection of Narcoren™ (Pentobarbital Sodium, Rhone Merieux). The lungs were then removed, blotted dry and half was snap frozen in liquid nitrogen and stored at -80°C. The other half was fixed in 4% formalin for subsequent paraffin embedding and histology.

Histology

[0023] The lung tissues fixed in 4% formalin were embedded into paraffin and 5µm sections were cut using a microtome (Leica SM200R) and placed on poly-L-lysine coated slides. The sections were then dried onto the slides (60°C 2 hours) and then left to cool at room temperature. Collagen deposition was assessed using Masson’s Trichrome staining.

Results

[0024] Figure 1A shows the result obtained with the control group, which received saline and the vehicle instead of bleomycin intratracheally.

[0025] Rats treated intratracheally with bleomycin and the vehicle developed severe lung fibrosis, as seen in Figure 1B. The alveoli have been largely replaced by fibroblasts and extracellular matrix and the normal lung structure is nearly obliterated.

[0026] Daily treatment of bleomycin-treated rats with 50 mg/kg of Example A (compound (m)) showed a consistent, nearly complete reversal of lung fibrosis in this model. A typical example is shown in Figure 1C. Alveoli are intact and little or no fibroblast infiltration or extracellular matrix deposition has occurred. Normal lung structure has been maintained, which is evidenced by a comparison of Figure 1C with Figure 1A.

[0027] (B) Effect on expression of fibrotic marker genes following bleomycin-induced pulmonary fibrosis.
mRNA extractions and synthesis of cDNA

[0028] One part of the frozen lung tissue dedicated to investigation of gene expression was cut into small pieces using a sterile scalpel blade. Approximately 100mg of tissue was then placed into a 2ml Eppendorf tube and 1.5ml of Trizol (Invitrogen) was added. A sterile tungsten carbide bead (Qiagen) was then added to the tube and the tube was placed in a Retsch MM300 Tissue disruptor (Qiagen) at a frequency of 30.0Hz for 8 minutes. After this time, the bead was removed and the sample centrifuged at 12000rpm for 10 minutes to remove tissue debris. The RNA was extracted using a modified version of the manufacturer's protocol supplied with Trizol. Briefly, 0.3ml chloroform was added to the tube and the tube shaken vigorously and then left to incubate at room temperature for 5 minutes, after which the tube was centrifuged for 15 minutes at 12000 rpm at 4°C. The upper colorless aqueous phase was then collected and added to 750μl isopropanol. This was then shaken vigorously and stored at -80°C overnight. The samples were then incubated at room temperature for 15 minutes, after which they were centrifuged for 40 minutes at 12000 rpm at 4°C. The supernatant was then removed and 500μl of 70% ethanol was added to wash the pellet then the sample was centrifuged for 10 minutes at 12000 rpm an 4°C, this wash step was repeated twice, after which the pellet was left to dry for 10 - 15 minutes. Finally the pellet was resuspended in 20μl RNase free water and stored at -80°C. The concentration of each sample was then measured using a spectrophotometer.

[0029] Using the Superscript™ III (Invitrogen, Paisley, UK) RT-first strand synthesis kit, 2μg of each mRNA sample was reversed transcribed using a modified version of the manufacturer's protocol. Briefly, a mixture of 2μg RNA, 1μl random hexamer primers (50ng/μl), 1μl dNTP mix (10mM) was made up to 10μl with DEPC-treated water and incubated at 65°C for 5 minutes, after which it was placed on ice for 5 minutes. Following this, to each reaction, 2μl RT buffer (10X), 4μl MgCl₂ (25mM), 2μl DTT (0.1M), 1μl RNaseOUT™ (40U/μl) and 1μl SuperScript™ III enzyme (200U/μl) was added and the mixture placed in a thermal cycler (Applied Biosystems) under the following conditions: 25° C for 10 minutes, 50°C for 50 minutes and 85°C for 5 minutes, after which 1μl of RNase H was added and incubated at 37°C for 20 minutes. The synthesized cDNA was diluted to 5ng/μl using the assumption that the RT reaction fully transcribed all of the mRNA to cDNA and was a concentration of 100ng/μl.

Investigation of gene expression using real time PCR

[0030] Gene expression was investigated in each of the samples using the Applied Biosystems 7700 sequence detection system. Primers for the 18S endogenous control and TGFβ1 were purchased as pre-developed assay reagent kits, whereas primers and probes (see Table 2 below) for pro-collagen I and fibronectin were designed using PrimerExpress™ (Applied Biosystems), ensuring that at least one of the primers or probes in each set overlapped an intron / exon junction, thus eliminating the possibility of amplifying any contaminating genomic DNA in the cDNA sample. The purchased PDARs also amplified only cDNA.

| Table 2 |
|---|---|
| **Target** | **Sequence** |
| Fibronectin | **Forward** 5’-GAT GCC GAT CAG AAG TTT GGA-3’ |
|       | **Reverse** 5’-TCG TTG GTC GTG CAG ATC TC-3’ |
|       | **Probe** 5’-FAM-CTG CCC AAT GGC TGC CCA TGA-TAMRA-3’ |
| Pro-Collagen I | **Forward** 5’-CAG ACT GGC AAC CTG AAG AAG TC-3’ |
|       | **Reverse** 5’-TCG CCC CTG AGC TCG AT-3’ |
|       | **Probe** 5’-FAM-CTG CTC CTC CAG GCC TCC AAC GA-TAMRA3’ |
Real Time PCR was carried out in 25μl reactions, using 25ng (5μl) of cDNA per reaction. A quantitative PCR core kit was purchased (Eurogentec) and a master-mix was made up as follows for 100 reactions: 500μl 10X reaction buffer, 500μl MgCl2 (50mM), 200μl dNTP mix solution (5mM), 25μl Hot Goldstar enzyme, 75μl 18S PDAR, 22.5μl forward primer, 22.5μl reverse primer, 15μl probe and 640μl DEPC treated water. 20μl of this master-mix was then added to 25ng (5μl) target cDNA. Each analysis was carried out in triplicate.

In order to quantify the gene expression, a standard curve was constructed for each primer set and was included on each plate. The standards were made up of a mix of all the cDNA’s under investigation; this mix of cDNA’s was serially diluted 10, 20, 50, 100, 100 times. A standard curve was constructed of the obtained C_T (Cycle at which amplification reaches a set Threshold) against the LOG_{10} of the dilution factor. Curves were drawn for the target gene and the 18S rRNA endogenous control. The C_T value for both targets for each of the samples was then converted to a fold dilution using the standard curve and the target gene value was normalized to the 18S gene value.

All statistical analyses were carried out using GraphPad Prism V 4.02 software. Comparisons were made using a non-parametric T-test (Mann-Whitney U test) and a significant value was considered to be p = 0.05.

The results are shown in Figures 2 (procollagen I) and 3 (fibronectin). Each data point represents RNA isolated from the lung of a single rat.

Intratracheal administration of bleomycin and subsequent treatment with vehicle only showed large increases in procollagen I and fibronectin gene expression in the lung, as seen in Figures 2 and 3, consistent with the histologically apparent lung fibrosis seen in Figure 1B.

Daily treatment of Bleomycin-treated rats with 50 mg/kg of Example A (compound (m)) showed a significant (p ≤ 0.0001) inhibition of expression of fibrotic marker genes in this model, as seen in Figures 2 and 3.

This experiment thus demonstrates that expression of fibrotic markers, and therefore deposition of extracellular matrix, may be dramatically reduced by treatment with Example A (compound (m)).

In the following experiments of Example B2, the compound 3-Z-[1-(4-(N-((4-methyl-piperazin-1-yl)-methylcarbonyl)-N-methyl-amino)-anilino)-1-phenyl-methylene]-6-methoxycarbonyl-2-indolinone, which is compound (u), was used.

All the methods employed are the same as the methods described in the experiments of Example B1, however using compound (u) instead of compound (m).

(A) Effect on lung morphology following bleomycin-induced pulmonary fibrosis.

Samples were prepared from rats treated as outlined in above Table 1 of experimental Example B1 (A).

Figure 4A shows the result obtained with the control group, which received saline and the vehicle instead of bleomycin intratracheally.

Rats treated intratracheally with bleomycin and the vehicle developed severe lung fibrosis, as seen in Figure 4B. The alveoli have been largely replaced by fibroblasts and extracellular matrix and the normal lung structure is nearly obliterated.

Daily treatment of bleomycin-treated rats with 50 mg/kg of compound (u) showed a consistent, nearly complete reversal of lung fibrosis in this model. A typical example is shown in Figure 4C. Alveoli are intact and little or no fibroblast infiltration or extracellular matrix deposition has occurred. Normal lung structure has been maintained, which is evidenced by a comparison of Figure 4C with Figure 4A.

(B) Effect on expression of fibrotic marker genes following Bleomycin-induced pulmonary fibrosis.

The experiment was carried out using the methods as outlined above for Example B1 (B). The results are shown in Figure 5 (procollagen I) and Figure 6 (TGFβ). Each data point represents RNA isolated from the lung of a single rat.

Intratracheal administration of bleomycin and subsequent treatment with vehicle only showed large increases in procollagen I and TGFβ gene expression in the lung, as seen in Figures 5 and 6, consistent with the histologically apparent lung fibrosis seen in Figure 1B.

Daily treatment of bleomycin-treated rats with 50 mg/kg of (compound (u) showed a significant (p ≤ 0.0001)
inhibition of expression of fibrotic marker genes in this model, as seen in Figures 5 and 6.

[0050] This experiment also demonstrates that expression of fibrotic markers, and therefore deposition of extracellular matrix, may be dramatically reduced by treatment with compound (u).

[0051] By reason of its biological properties the compound according to the invention may be used in monotherapy or in conjunction with other pharmaceutically active compounds. Such pharmaceutically active compounds may be compounds which are, for example, also pharmaceutically active in the treatment of fibrosis. Such pharmaceutically active compounds may also be substances with a secretolytic, broncholytic and/or anti-inflammatory activity.

[0052] In a preferred embodiment in accordance with the present invention, such pharmaceutically active compounds are preferably selected from the group consisting of anticholinergic agents, beta-2 mimetics, steroids, PDE-IV inhibitors, p38 MAP kinase inhibitors, NK1 antagonists, LTD4 antagonists, EGFR inhibitors and endothelin-antagonists.

[0053] These combinations may be administered either simultaneously or sequentially.

[0054] For pharmaceutical use the compounds according to the invention are preferably used for warm-blooded vertebrates, particularly humans, in doses of 0.0001-100 mg/kg of body weight.

[0055] By reason of its biological properties the compound according to the invention may be used in monotherapy or in conjunction with other active substances by intravenous, subcutaneous, intramuscular, intraperitoneal or intranasal route, by inhalation, or transdermally, or orally, whilst aerosol formulations are particularly suitable for inhalation.

[0056] For administration they are formulated with one or more conventional inert solid, semisolid or liquid carriers e.g. with starch, different types of cellulose, lactose, mannitol, sorbitol, glucose, calcium phosphate, hard fat, fatty alcohols, glycerol, medium chained triglycerides and related esters, polyethylene glycol, refined specialty oils, water, water/ethanol, water/glycerol, water/sorbitol, water/polyethylene glycol, propylene glycol, and/or functional excipients, e.g. with polyvinylpyrrolidone, hydroxypropylmethylcellulose, sodium carboxymethylcellulose, sodium starch glycolate, silicon dioxide, polysorbates, poloxamers, gelucires, magnesium stearate, citric acid, tartaric acid, or suitable mixtures thereof in conventional galenic preparations such as plain or coated tablets, capsules, powders, injectable solutions, ampoules, suspensions, solutions, sprays or suppositories.

[0057] The following examples of formulations illustrate the present invention without representing a limitation of its scope.

Example F1: Coated tablet containing 75 mg of active substance

Composition

[0058] active substance 75.0 mg
calcium phosphate 131.0 mg
polyvinylpyrrolidone 10.0 mg
carboxymethylcellulose sodium 10.0 mg
silicon dioxide 2.5 mg
magnesium stearate 1.5 mg

230.0 mg

Preparation (direct compression)

[0059] The active substance is mixed with all components, sieved and compressed in a tablet-making machine to form tablets of the desired shape.

Weight of core: 230 mg
Appearance of core: 9 mm, biconvex

[0060] The tablet cores thus produced are coated with a film consisting essentially of hydroxypropylmethylcellulose.

Weight of coated tablet: 240 mg.
Example F2: Tablet containing 100 mg of active substance

Composition

1 tablet contains:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>active substance</td>
<td>100.0</td>
</tr>
<tr>
<td>lactose</td>
<td>80.0</td>
</tr>
<tr>
<td>corn starch</td>
<td>34.0</td>
</tr>
<tr>
<td>hydroxypropylmethylcellulose</td>
<td>4.0</td>
</tr>
<tr>
<td>magnesium stearate</td>
<td>2.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>220.0</strong></td>
</tr>
</tbody>
</table>

Preparation (wet granulation)

[0062] The active substance, lactose and starch are mixed together and uniformly moistened with an aqueous solution of the hydroxypropylmethylcellulose. After the moist composition has been screened (2.0 mm mesh size) and dried in a rack-type drier at 50°C it is screened again (1.5 mm mesh size) and the lubricant is added. The finished mixture is compressed to form tablets.

Weight of tablet: 220 mg
Appearance of tablet: 10 mm, flat faced

with bevelled edges and breaking notch on one side.

Example F3: Tablet containing 150 mg of active substance

Composition

1 tablet contains:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>active substance</td>
<td>150.0</td>
</tr>
<tr>
<td>lactose</td>
<td>85.0</td>
</tr>
<tr>
<td>microcrystalline cellulose</td>
<td>40.0</td>
</tr>
<tr>
<td>polyvinylpyrrolidone</td>
<td>10.0</td>
</tr>
<tr>
<td>silicon dioxide</td>
<td>10.0</td>
</tr>
<tr>
<td>magnesium stearate</td>
<td>5.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>300.0</strong></td>
</tr>
</tbody>
</table>

Preparation (dry granulation)

[0064] The active substance mixed with lactose, polyvinylpyrrolidone, and parts of the microcrystalline cellulose, magnesium stearate is compacted e.g. on a roller compactor. The ribbons are broken up in fine granules through a screen with a mesh size of 0.8 mm. After subsequent sieving through a screen with a mesh size of 0.5 mm and blending with the remaining components, tablets are pressed from the mixture.

Weight of tablet: 300 mg
Appearance of tablet: 10 mm, flat
Example F4: Hard gelatine capsule containing 150 mg of active substance

Composition

1 capsule contains:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>active substance</td>
<td>150.0 mg</td>
</tr>
<tr>
<td>lactose</td>
<td>85.0 mg</td>
</tr>
<tr>
<td>microcrystalline cellulose</td>
<td>40.0 mg</td>
</tr>
<tr>
<td>polyvinylpyrrolidone</td>
<td>10.0 mg</td>
</tr>
<tr>
<td>silicon dioxide</td>
<td>10.0 mg</td>
</tr>
<tr>
<td>magnesium stearate</td>
<td>5.0 mg</td>
</tr>
<tr>
<td></td>
<td>300.0 mg</td>
</tr>
</tbody>
</table>

Preparation

The active substance mixed with lactose, polyvinylpyrrolidone, and parts of the microcrystalline cellulose, magnesium stearate is compacted e.g. on a roller compactor. The ribbons are broken up in fine granules through a screen with a mesh size of 0.8 mm. After subsequent sieving through a screen with a mesh size of 0.5 mm and blending with the remaining components, the finished mixture is packed into size 1 hard gelatine capsules.

Capsule filling: approx. 300 mg
Capsule shell: size 1 hard gelatine capsule.

Example F5: Suppository containing 150 mg of active substance

1 suppository contains:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>active substance</td>
<td>150.0 mg</td>
</tr>
<tr>
<td>polyethyleneglycol 1500</td>
<td>800.0 mg</td>
</tr>
<tr>
<td>polyethyleneglycol 6000</td>
<td>850.0 mg</td>
</tr>
<tr>
<td>polyoxyl 40 hydrogenated castor oil</td>
<td>200.0 mg</td>
</tr>
<tr>
<td></td>
<td>2,000.0 mg</td>
</tr>
</tbody>
</table>

Preparation

After the suppository mass has been melted the active substance is homogeneously distributed therein and the melt is poured into chilled moulds.

Example F6: Suspension containing 50 mg of active substance

100 ml of suspension contains

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>active substance</td>
<td>1.00 g</td>
</tr>
<tr>
<td>carboxymethylcellulose sodium</td>
<td>0.10 g</td>
</tr>
<tr>
<td>methyl p-hydroxybenzoate</td>
<td>0.05 g</td>
</tr>
<tr>
<td>propyl p-hydroxybenzoate</td>
<td>0.01 g</td>
</tr>
<tr>
<td>glucose</td>
<td>10.00 g</td>
</tr>
<tr>
<td>glycerol</td>
<td>5.00 g</td>
</tr>
<tr>
<td>70% sorbitol solution</td>
<td>20.00 g</td>
</tr>
</tbody>
</table>
Preparation

[0070] The distilled water is heated to 70°C. The methyl and propyl p-hydroxybenzoates together with the glycerol and sodium salt of carboxymethylcellulose are dissolved therein with stirring. The solution is cooled to ambient temperature and the active substance is added and homogeneously dispersed therein with stirring. After the sugar, the sorbitol solution and the flavouring have been added and dissolved, the suspension is evacuated with stirring to eliminate air.

[0071] Thus, 5 ml of suspension contains 50 mg of active substance.

Example F7: Ampoule containing 10 mg active substance

Composition

[0072]

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>active substance</td>
<td>10.0 mg</td>
</tr>
<tr>
<td>0.01 N hydrochloric acid</td>
<td>q.s.</td>
</tr>
<tr>
<td>double-distilled water</td>
<td>ad 2.0 ml</td>
</tr>
</tbody>
</table>

Preparation

[0073] The active substance is dissolved in the necessary amount of 0.01 N HCl, made isotonic with sodium chloride, filtered sterile and transferred into a 2 ml ampoule.

Example F8: Ampoule containing 50 mg of active substance

Composition

[0074]

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>active substance</td>
<td>50.0 mg</td>
</tr>
<tr>
<td>0.01 N hydrochloric acid</td>
<td>q.s.</td>
</tr>
<tr>
<td>double-distilled water</td>
<td>ad 10.0 ml</td>
</tr>
</tbody>
</table>

Preparation

[0075] The active substance is dissolved in the necessary amount of 0.01 N HCl, made isotonic with sodium chloride, filtered sterile and transferred into a 10 ml ampoule.

Example F9: Capsule for powder inhalation containing 5 mg of active substance

1 capsule contains

[0076]

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>active substance</td>
<td>5.0 mg</td>
</tr>
<tr>
<td>lactose for inhalation</td>
<td>15.0 mg</td>
</tr>
<tr>
<td></td>
<td>20.0 mg</td>
</tr>
</tbody>
</table>

Preparation

[0077] The active substance is mixed with lactose for inhalation. The mixture is packed into capsules in a capsule-
Example F10: Solution for inhalation for a hand-held nebuliser containing 2.5 mg active substance

1 spray contains

active substance 2.500 mg
benzalkonium chloride 0.001 mg
1N hydrochloric acid q.s.
ethanol/water (50/50) ad 15,000 mg

Preparation

[0078] The active substance and benzalkonium chloride are dissolved in ethanol/water (50/50). The pH of the solution is adjusted with 1N hydrochloric acid. The resulting solution is filtered and transferred into suitable containers for use in hand-held nebulisers (cartridges).
Contents of the container: 4.5 g

Claims

1. Compound 3-Z-[1-(4-((4-methyl-piperazin-1-yl)-methylcarbonyl)-N-methyl-amino)-anilino]-1-phenylmethylene]-6-methoxycarbonyl-2-indolinone or a tautomer, a diastereomer, an enantiomer, the mixtures thereof or a salt thereof, for use in the prevention or treatment of idiopathic pulmonary fibrosis.

2. Monoethanesulfonate salt of the compound 3-Z-[1-(4-((4-methyl-piperazin-1-yl)-methylcarbonyl)-N-methyl-amino)-anilino]-1-phenylmethylene]-6-methoxycarbonyl-2-indolinone, for use in the prevention or treatment of idiopathic pulmonary fibrosis according to claim 1.

Revendications

1. Composé 3-Z-[1-(4-((4-méthyl-pipérazin-1-yl)-méthylcarbonyl)-N-méthyl-amino)-anilino]-1-phénylméthylène]-6-méthoxycarbonyl-2-indolinone ou un tautomère, un diastéréoisomère, ou un énantiomère de celui-ci, des mélanges de ceux-ci ou un sel de celui-ci, pour leur utilisation dans la prévention ou le traitement d’une fibrose pulmonaire idiopathique.

2. Sel monoéthanesulfonate du composé 3-Z-[1-(4-((4-méthyl-pipérazin-1-yl)-méthylcarbonyl)-N-méthyl-amino)-anilino]-1-phénylméthylène]-6-méthoxycarbonyl-2-indolinone, pour son utilisation dans la prévention ou le traitement d’une fibrose pulmonaire idiopathique selon la revendication 1.
FIGURE 2

![Gene Expression Graph](image)

- Saline
- Bleomycin (10 U/kg)
- Bleo + Example A

$p \leq 0.0001$
REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader’s convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- WO 0127081 A
- WO 0413099 A

Non-patent literature cited in the description

- JINNIN et al. J Cell Physiol, 2004
- JELASKA et al. Springer Semin Immunopathol, 2000, vol. 21, 385-95
- BORKHAM-KAMPHORST et al. Biochem Biophys Res Commun, 2004
- LAPIG. Curr Opin Pharmacol, 2003, vol. 3 (2), 204-8